

DR SAMANTHA S STUTZ (Orcid ID : 0000-0002-3999-9726)

PROF. DAVID T HANSON (Orcid ID : 0000-0003-0964-9335)

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**CONTRIBUTION AND CONSEQUENCES OF XYLEM-TRANSPORTED CO<sub>2</sub> ASSIMILATION FOR C<sub>3</sub> PLANTS**

Authors:

Samantha S. Stutz<sup>1, 2</sup>, David T. Hanson<sup>1</sup>

<sup>1</sup>Department of Biology, University of New Mexico, Albuquerque, NM 87131, USA

<sup>2</sup>Present address: Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana, Illinois 61801, USA

Author for correspondence: Samantha Stutz

Telephone: +1 (505) 690-2609

Email: samstutz@illinois.edu

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## Summary

- Traditionally, leaves were thought to be supplied with CO<sub>2</sub> for photosynthesis by the atmosphere and respiration. Recent studies, however, have shown that the xylem also transports a significant amount of inorganic carbon into leaves by the bulk flow of water. However, little is known about the dynamics and proportion of xylem-transported CO<sub>2</sub> that is assimilated, versus simply lost to transpiration.
- Cut leaves of *Populus deltoides* and *Brassica napus* were placed in either KCl or one of three [NaH<sup>13</sup>CO<sub>3</sub>] solutions dissolved in water to simultaneously measure the assimilation and the efflux of xylem-transported CO<sub>2</sub> exiting the leaf across light- and CO<sub>2</sub>-response curves in real-time using a tunable diode laser absorption spectroscopy.
- The rates of assimilation and efflux of xylem-transported CO<sub>2</sub> increased with increasing xylem [<sup>13</sup>CO<sub>2</sub><sup>\*</sup>] and transpiration. Under saturating irradiance, rates of assimilation using xylem-transported CO<sub>2</sub> accounted for ~2.5% of the total assimilation in both species in the highest [<sup>13</sup>CO<sub>2</sub><sup>\*</sup>].
- The majority of xylem-transported CO<sub>2</sub> is assimilated, and efflux is small compared to respiration. Assimilation of xylem-transported CO<sub>2</sub> comprises a small portion of total photosynthesis, but may be more important when CO<sub>2</sub> is limiting.

Key words: *Brassica napus*, CO<sub>2</sub> efflux, internally transported CO<sub>2</sub>, leaf photosynthesis models, *Populus deltoides*, stem [CO<sub>2</sub><sup>\*</sup>], tunable diode laser absorption spectroscopy, xylem-transported CO<sub>2</sub>

## INTRODUCTION

Traditionally, aside from mitochondrial respiration in the same cell or immediately adjacent cells, CO<sub>2</sub> for photosynthesis was assumed to diffuse from the atmosphere through the stomata into the intercellular air space. This CO<sub>2</sub> then diffuses into the mesophyll cells eventually making it to the site of ribulose-1,5-bisphosphate-carboxylase/oxygenase (Rubisco) in the chloroplast where it will be used for photosynthesis. However, recent work has shown that the concentration of total dissolved inorganic carbon ([CO<sub>2</sub><sup>\*</sup>], the sum of [CO<sub>2</sub>]<sub>aq</sub>, [HCO<sub>3</sub><sup>-</sup>], [H<sub>2</sub>CO<sub>3</sub>], and [CO<sub>3</sub><sup>2-</sup>]) in the xylem ranges from ~0.05 to ~13 mmol l<sup>-1</sup> (<1 to 26% CO<sub>2</sub> gas by volume in air equilibrated with xylem sap). These levels are ~30-750 times higher than expected based on current atmospheric [CO<sub>2</sub>] (Teskey *et al.*, 2008). Dissolved CO<sub>2</sub> in the stem is derived from root and stem respiration and moves in bulk flow along with water through the plant to the leaf where it is either recaptured or exits via transpiration (Fig.

S1). If the concentration of xylem-transported CO<sub>2</sub> is high and reaches the foliage it could provide a major substrate for Rubisco in leaf cells; thus changing the calculations and understanding of the relationship between assimilation and intercellular [CO<sub>2</sub>] (C<sub>i</sub>) in the Farquhar *et al.* (1980) model of leaf photosynthesis (Hanson & Gunderson, 2009). However, if little xylem-transported CO<sub>2</sub> is recaptured by photosynthesis, xylem-transported CO<sub>2</sub> exiting the leaf in the light may account for some of the variation seen in estimates of respiration in the light.

Previous studies have shown that plants utilize xylem-transported CO<sub>2</sub> for photosynthesis by corticular and woody stem tissue (Teskey *et al.*, 2008), by branch tissue (Teskey & McGuire, 2002; McGuire *et al.*, 2009; Bloemen *et al.*, 2013a, b), and by leaves (Stringer & Kimmerer, 1993; McGuire *et al.*, 2009; Bloemen *et al.*, 2013a, b; Bloemen *et al.*, 2015). Cut *Platanus occidentalis* branches assimilated ~35% of xylem-transported CO<sub>2</sub> when supplied with 11.9 mmol l<sup>-1</sup> <sup>13</sup>CO<sub>2</sub> \* (McGuire *et al.*, 2009). Over half of the assimilated xylem-transported CO<sub>2</sub> was incorporated into the woody branch tissues and nearly a third into the leaves (McGuire *et al.*, 2009). A similar study estimated that assimilation of xylem-transported CO<sub>2</sub> represented ~2% of net total photosynthesis in *Populus deltoides* (Bloemen *et al.*, 2013a). These studies demonstrate that xylem-transported CO<sub>2</sub> taken up by a cut branch will make it to the leaves where it is used for photosynthesis and generate similar results to those where individual leaves were supplied label through the petiole (Stringer & Kimmerer, 1993; Bloemen *et al.*, 2015). However, when saplings were supplied labeled inorganic carbon through the base of their stems less of the xylem-transported CO<sub>2</sub> was incorporated into the above-ground portions of the plant (Bloemen *et al.*, 2013b), presumably due to the longer path-length through the plant. Since the percent captured declines at the highest concentrations of xylem-supplied <sup>13</sup>C (Bloemen *et al.*, 2013a), it appears that conditions leading to high rates of xylem-transported CO<sub>2</sub> supply also lead to greater rates of loss relative to rates of re-capture by photosynthesis.

If this flux of xylem-transported CO<sub>2</sub> out of leaves is large, it may account for some of the variation observed in rates of day respiration. Current models of leaf photosynthesis incorporate CO<sub>2</sub> evolution from the mitochondria in the light not associated with photorespiration (R<sub>d</sub>) (Farquhar *et al.*, 1980); additionally, models assume that all fluxes of CO<sub>2</sub> exiting a leaf are derived from metabolism occurring in leaf cells. While it is generally thought that rates of respiration are lower in the light compared to the dark (Kok, 1948, 1949; Tcherkez *et al.*, 2017a), it is difficult to measure rates of respiration in the light while leaves are photosynthesizing. Variation in the inhibition of leaf respiration in the light may be complicated by an efflux of xylem-transported CO<sub>2</sub> exiting the leaf. For instance, when rates of assimilation are low, as occurs under low irradiance or low CO<sub>2</sub>, the

overall proportion of day respiration is greater than when rates of assimilation are high, as occurs under high irradiance and high CO<sub>2</sub> (Tcherkez *et al.*, 2017b). If the efflux of xylem-transported CO<sub>2</sub> out of a leaf is large when irradiance or [CO<sub>2</sub>] are low, then it could explain this observation as well as a portion of the Kok effect (Kok, 1949). Although earlier work showed fixation of xylem-transported CO<sub>2</sub> varied with light intensity (Stringer & Kimmerer, 1993) no study has demonstrated the dynamics of the efflux and capture of xylem-transported CO<sub>2</sub> exiting a leaf in the light with respect to simultaneous measurements of stomatal conductance and transpiration.

Radiocarbon or mass spectroscopy methods also required destructive sampling and allow only a snapshot at a discrete point in time rather than real-time repeated measures on a single leaf across a range of environmental conditions. Therefore, the objectives of this study are to: 1) determine how much and under what conditions xylem-transported CO<sub>2</sub> is most important for leaf photosynthesis in excised leaves, 2) determine if the concentration of xylem-transported CO<sub>2</sub> changes the modeling parameters for leaf-level photosynthesis, 3) determine how much xylem-transported CO<sub>2</sub> exits (<sup>13</sup>C<sub>light efflux</sub>) a leaf in the light, and 4) if <sup>13</sup>C<sub>light efflux</sub> has the potential to change estimates of day respiration in excised leaves of a woody and a herbaceous C<sub>3</sub> plant. These objectives were accomplished by adding one of three [NaH<sup>13</sup>CO<sub>3</sub>] solutions to cut leaves of *P. deltoides* and *Brassica napus* and measuring rates of <sup>12</sup>C and <sup>13</sup>C assimilation and the efflux of xylem-transported CO<sub>2</sub> exiting the leaf across light- and CO<sub>2</sub>-response curves in real-time using a tunable diode laser absorption spectroscopy (TDL). We hypothesized that the rates of assimilation using xylem-transported CO<sub>2</sub> (<sup>13</sup>A<sub>x</sub>) would be greatest when intercellular [CO<sub>2</sub>] was low and that the <sup>13</sup>C<sub>light efflux</sub> would be highest when rates of transpiration were high.

## MATERIALS & METHODS

### *Plant propagation and growth*

*Brassica napus* (L. stellar DH GT060615) and *Populus deltoides* (W. Bartram ex Marshall) were propagated and grown according to Stutz *et al.* (2017). All plants were grown under natural light in an unshaded greenhouse, with mid-day photosynthetically active radiation (PAR) at pot level of approximately 1200 μmol m<sup>-2</sup> s<sup>-1</sup> at the University of New Mexico in Albuquerque, NM, USA, under ambient CO<sub>2</sub>, 24°C/21°C day/night. *B. napus* and *P. deltoides* were fertilized twice weekly with Peters 20-20-20 fertilizer (Scotts Miracle-Gro, Marysville, OH, USA) and once weekly with chelated liquid iron (ferti-lome, Bonham, TX, USA). *B. napus* plants were measured between 14 and 25 days after germinating.

### Light-response curves

A LI-6400 (LI-COR Biosciences, Lincoln, NE, USA) was coupled to a tunable diode laser absorption spectroscopy (TDL—model TGA 100; Campbell Scientific, Inc., Logan, UT, USA) to measure online  $^{12}\text{CO}_2$  and  $^{13}\text{CO}_2$  exchange. Isotope calibration consisting of a high and low  $\text{CO}_2$  tank spanning the expected range of  $[\text{CO}_2]$  of each isotopologue for the LI-COR reference and sample was completed as previously described in Barbour *et al.* (2007) and Stutz *et al.* (2017). The highest fully expanded leaf for *B. napus* or a fully expanded *P. deltoides* leaf was placed in a clear topped, custom leaf chamber  $38.5\text{ cm}^2$  made to fit an RGB LED light source (LI-6400-18, LI-COR Biosciences, Lincoln, NE, USA) attached to a LI-6400 at  $23^\circ\text{C}$  (leaf temperature),  $380\text{ }\mu\text{mol mol}^{-1}\text{ CO}_2$  reference at  $1200\text{ }\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$  or  $1500\text{ }\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$  for *B. napus* and *P. deltoides*, respectively. This large leaf chamber (roughly six times large than a standard LI-COR leaf chamber) was used to ensure the fluxes of each  $\text{CO}_2$  isotopologue were large enough to be easily measured by the TDL; the standard deviation of empty chambers were  $\sim 0.1\text{ ppm}$  and  $\sim 0.001\text{ ppm}$  for  $^{12}\text{CO}_2$  and  $^{13}\text{CO}_2$ , respectively. Once photosynthesis reached a steady state ( $\sim 30$  minutes), the leaf was detached from the plant and the petiole placed in a  $40\text{ mmol l}^{-1}\text{ KCl}$  solution. The KCl solution was swapped for  $99\%\text{ }^{13}\text{C}$  sodium bicarbonate ( $\text{NaH}^{13}\text{CO}_3$ —Cambridge Isotope Laboratories, Inc. Andover, MA, USA) dissolved in  $40\text{ mmol l}^{-1}\text{ KCl}$  at one of three  $^{13}\text{CO}_2^*$ :  $1.19$  (low-carbon—LC),  $5.95$  (medium-carbon—MC), or  $11.9$  (high-carbon—HC)  $\text{mmol l}^{-1}\text{ }^{13}\text{CO}_2^*$ , or the leaf was left in the KCl solution for the measurement. Individual leaves were provided a single  $^{13}\text{CO}_2^*$  or left in the  $40\text{ mmol l}^{-1}\text{ KCl}$  solution according to Stutz *et al.* (2017). Once cut and placed in the  $^{13}\text{CO}_2^*$  the leaf remained at the starting irradiance ( $1200\text{ }\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$  *B. napus* or  $1500\text{ }\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$  *P. deltoides*) until  $\delta^{13}\text{C}$  and  $^{13}\text{CO}_2$  peaked and either plateaued or decreased, which took approximately 20–30 minutes, before continuing with the light-response curve. The light-response curves were measured in the following order for *B. napus*: 1200, 1000, 800, 500, 350, 250, 200, 175, 150, 125, 100, 75, 50, 35,  $0\text{ }\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$  and the following order for *P. deltoides*: 1500, 1000, 800, 500, 250, 200, 175, 150, 125, 100, 75, 50, 35,  $0\text{ }\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$ . Five measurements were made at each irradiance. Five leaves from both species were measured in the KCl and each  $^{13}\text{CO}_2^*$ .

### $\text{CO}_2$ -response curves

For the  $\text{CO}_2$ -response curves, the highest fully expanded leaf for *B. napus* or a fully expanded *P. deltoides* leaf, was placed in the same LI-6400 custom leaf chamber as was used for the light-response curves at  $23^\circ\text{C}$  (leaf temperature) at  $1200\text{ }\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$  or  $1500\text{ }\mu\text{mol quanta m}^{-2}\text{ s}^{-1}$

for *B. napus* and *P. deltoides*, respectively. The CO<sub>2</sub> reference on the LI-6400 was set so the [CO<sub>2</sub>] at the leaf surface was approximately 400 μmol mol<sup>-1</sup>, which was between 500 and 600 μmol mol<sup>-1</sup> CO<sub>2</sub> reference [CO<sub>2</sub>] for both species. As with the light-response curves, attached leaves were left in the chamber for approximately 30 minutes before the petiole was cut and placed in a 40 mmol l<sup>-1</sup> KCl solution for ~16 minutes. The leaves were then transferred to a single 99% <sup>13</sup>C sodium bicarbonate (NaH<sup>13</sup>CO<sub>3</sub>) solution (LC, MC, or HC) or remained in the KCl solution, as in the light-response curves. The leaf was left at the starting [CO<sub>2</sub>] until the δ<sup>13</sup>C and [<sup>13</sup>CO<sub>2</sub>] readings on the TDL stabilized approximately 20 to 30 minutes for each leaf. The [CO<sub>2</sub>] on the LI-6400 reference were applied to the leaf in the following order: starting [CO<sub>2</sub>], 200, 100, 50, 150, 300, starting [CO<sub>2</sub>], 1000, 2000, 1500, 700 and starting [CO<sub>2</sub>]. The leaf was left at each [CO<sub>2</sub>] for three cycles on the TDL except for the starting [CO<sub>2</sub>] where the leaf was left until it reached the starting rate of photosynthesis. Following the CO<sub>2</sub>-response curve, the light was turned off and respiration was measured when the leaf had been in the dark for at least 30 minutes, to avoid light enhanced dark respiration (LEDR). Five leaves from both species were measured in the KCl and each [<sup>13</sup>CO<sub>2</sub>\*].

#### ***Estimating the rate of xylem-transported CO<sub>2</sub> assimilation***

The predicted rate of net CO<sub>2</sub> assimilation using <sup>13</sup>CO<sub>2</sub> (<sup>13</sup>A<sub>pred</sub>) was calculated as (Table 1, Fig. S2):

$$^{13}A_{pred} = ^{12}A_{obs} * 0.011/0.989 \quad \text{Equation 1}$$

where <sup>12</sup>A<sub>obs</sub> is the observed rate of net <sup>12</sup>CO<sub>2</sub> assimilation measured with the TDL. The natural abundance of <sup>13</sup>CO<sub>2</sub> and <sup>12</sup>CO<sub>2</sub> are approximately 1.1% and 98.9% of total CO<sub>2</sub>, respectively.

Therefore, the rate of <sup>13</sup>CO<sub>2</sub> assimilation is approximately 1.1% of the rate of <sup>12</sup>CO<sub>2</sub> assimilation (0.011/0.989) under normal conditions (Griffis *et al.*, 2004).

The efflux of xylem-transported <sup>13</sup>CO<sub>2</sub> exiting the leaf in the light (<sup>13</sup>C<sub>light efflux</sub>) is calculated as (Table 1, Fig. 1, Fig. S2):

$$^{13}C_{light\ efflux} = ^{13}A_{pred} - ^{13}A_{obs} \quad \text{Equation 2}$$

where <sup>13</sup>A<sub>obs</sub> is the rate of net <sup>13</sup>CO<sub>2</sub> assimilation measured with the TDL.

The rate of net assimilation of xylem-transported CO<sub>2</sub> (<sup>13</sup>A<sub>x</sub>) is thus calculated as (Table 1, Fig. 1, Fig. S3):

$$^{13}A_x = ^{13}C_{pred\ efflux} - ^{13}C_{light\ efflux} \text{ Equation 3}$$

where  $^{13}C_{pred\ efflux}$  (Table 1) is the predicted rate of xylem-transported  $CO_2$  exiting the leaf in the absence of assimilation of xylem-transported  $CO_2$ .  $^{13}C_{pred\ efflux}$  was estimated by generating linear regression models for the LC, MC and HC treatments for *B. napus* and *P. deltoides*, by plotting the  $^{13}CO_2$  efflux in the dark ( $^{13}C_{dark\ efflux}$ ) measured with the TDL against the rate of transpiration (Stutz *et al.*, 2017).

### Statistical analysis

For the light- and  $CO_2$ -response curves, we used the lme4 R package (Bates *et al.*, 2012) to perform linear mixed effects analyses of the relationship between our physiological response variables ( $^{12}A_{obs}$ ,  $^{13}A_x$ ,  $^{13}C_{light\ efflux}$  and the percentage of  $^{13}A_x$  to total A), species and [ $^{13}CO_2^*$ ]. We set species, [ $^{13}CO_2^*$ ] and irradiance or [ $CO_2$ ] in the light- and  $CO_2$ -response curves, respectively, as fixed effects. We structured the model to allow for random intercepts for individual leaves. Results were deemed significant at  $P < 0.05$ . No data were transformed based on the distribution of the residuals. All statistical analyses were performed in R (version 3.4.2, R Development Core Team 2017).

## RESULTS

### Efflux of xylem-transported $CO_2$ exiting the leaf in the light

Rates of efflux of xylem-transported  $CO_2$  in the light ( $^{13}C_{light\ efflux}$ ) change with light up to 250  $\mu mol\ quanta\ m^{-2}\ s^{-1}$  and then level off in both species (Fig. 2). In *B. napus*,  $^{13}C_{light\ efflux}$  was not significantly different among treatments (Fig. 2a, c, e). However, above the light-compensation point, the low-carbon (LC) treatment was significantly different from the medium-carbon (MC) and high-carbon (HC) treatments ( $P < 0.05$ ); while the MC and HC treatments were not significantly different across any irradiance.

In *P. deltoides*, there were significant differences in the  $^{13}C_{light\ efflux}$  between the LC and HC treatments ( $P < 0.001$ ) and between the LC and MC treatments ( $P < 0.05$ ) across all irradiances (Fig. 2b, d, f). Higher rates of  $^{13}C_{light\ efflux}$  were observed in leaves with higher transpiration rates within the MC and HC treatments compared to leaves with lower rates of transpiration within a single [ $^{13}CO_2^*$ ], leading to large error bars observed in *P. deltoides* (Fig. 2d, f). The  $^{13}C_{light\ efflux}$  was significantly different among [ $^{13}CO_2^*$ ] ( $P < 0.001$ ) but not between species ( $P = 0.43$ ). Differences in  $^{13}C_{light\ efflux}$  were

most pronounced under low irradiance both among [ $^{13}\text{CO}_2^*$ ] and between species (Fig. 2). The rate of  $^{13}\text{C}_{\text{light efflux}}$  was similar between species in the LC treatment; however maximum rates of  $^{13}\text{C}_{\text{light efflux}}$  were higher in *P. deltoides* compared to *B. napus* ( $P<0.001$ ) in the MC treatment (Fig. 2c, d); while rates of  $^{13}\text{C}_{\text{light efflux}}$  were higher in *B. napus* in the HC treatment compared to *P. deltoides* ( $P<0.001$ ) (Fig. 2e, f).

$^{13}\text{C}_{\text{light efflux}}$  increased slightly with increasing intercellular [ $\text{CO}_2$ ] for both species in the LC, and MC treatments and for *P. deltoides* in the HC treatment (Fig. 3a, b, c, d, f). However,  $^{13}\text{C}_{\text{light efflux}}$  decreased with increasing intercellular [ $\text{CO}_2$ ], in *B. napus*, in the HC treatment (Fig. 3e). There were significant differences between species ( $P<0.001$ ) and among [ $^{13}\text{CO}_2^*$ ] ( $P<0.001$ ).

### **Rates of assimilation**

As expected, net rates of assimilation using atmospheric  $\text{CO}_2$  ( $^{12}\text{A}_{\text{obs}}$ ) increased with increasing irradiance and intercellular [ $\text{CO}_2$ ] (Fig. 4c, d, Fig. 5c, d) for both species. Rates of xylem-transported  $\text{CO}_2$  assimilation ( $^{13}\text{A}_x$ ) also increased with increasing irradiance and [ $^{13}\text{CO}_2^*$ ] for both species (Fig. 4a, b). In *B. napus* saturating rates of  $^{13}\text{A}_x$  were significantly different among the three [ $^{13}\text{CO}_2^*$ ] ( $P<0.001$ ); rates of  $^{13}\text{A}_x$  and  $^{13}\text{A}_{\text{obs}}$  were significantly different ( $P<0.05$ ) in the MC and KCl treatments, respectively (Fig. 4a). In *P. deltoides*, under saturating irradiance, rates of  $^{13}\text{A}_x$  were significantly different among the three [ $^{13}\text{CO}_2^*$ ] ( $P<0.001$ ). However, in *P. deltoides*, rates of  $^{13}\text{A}_x$  and  $^{13}\text{A}_{\text{obs}}$  were not significantly different between the MC and the KCl treatments ( $P=0.063$ ) (Fig. 4b). Rates of  $^{13}\text{A}_{\text{obs}}$  in the KCl treatment were similar to the rates of  $^{13}\text{A}_x$  in the MC treatment for both species, indicating that under LC  $^{13}\text{A}_x$  is similar to the background rate of photosynthesis using  $^{13}\text{CO}_2$  derived from the atmosphere.

In the  $\text{CO}_2$ -response curves, rates of  $^{13}\text{A}_{\text{obs}}$  in the KCl treatment increased with increasing [ $\text{CO}_2$ ] and saturated at an intercellular [ $\text{CO}_2$ ] ( $C_i$ ) of  $\sim 700 \mu\text{mol mol}^{-1} \text{CO}_2$  for both species (Fig. 5a, b). However, in both species, across all [ $^{13}\text{CO}_2^*$ ], rates of  $^{13}\text{A}_x$  increased with decreasing [ $\text{CO}_2$ ] and peaked at an intercellular [ $\text{CO}_2$ ] of  $\sim 150 \mu\text{mol mol}^{-1}$  (Fig. 5a, b). In *B. napus*, rates of  $^{13}\text{A}_x$  and  $^{13}\text{A}_{\text{obs}}$  were significantly different among all treatments ( $P<0.001$ ). In *P. deltoides*, rates of  $^{13}\text{A}_x$  or  $^{13}\text{A}_{\text{obs}}$  were only significantly different in the HC treatment compared to the MC, LC and KCl treatments when the reference [ $\text{CO}_2$ ] was under  $400 \mu\text{mol mol}^{-1}$  ( $P<0.001$ ) (Fig. 5b). The saturating rate of  $^{13}\text{A}_{\text{obs}}$  in the KCl treatment was similar to the highest observed rate of  $^{13}\text{A}_x$  in the MC treatment; however, occurring at a different  $C_i$ . For both species, at an intercellular [ $\text{CO}_2$ ] of  $\sim 400 \mu\text{mol mol}^{-1}$  rates of  $^{13}\text{A}_x$

started to decline and continued to decline once saturation was reached for  $^{12}\text{CO}_2$ . Rates of  $^{13}\text{A}_x$  increased with  $[\text{}^{13}\text{CO}_2^*]$  and were significantly different among  $[\text{}^{13}\text{CO}_2^*]$  ( $P < 0.001$ ).

### **How do rates of $^{13}\text{A}_x$ compare to rates of $^{13}\text{C}_{\text{light efflux}}$ ?**

The rate of  $^{13}\text{C}_{\text{light efflux}}$  was lower than the rate of  $^{13}\text{A}_x$  across all irradiances in the LC and HC treatments, in *B. napus* (Fig. 2a, e). However, in the MC treatment, the rate of  $^{13}\text{C}_{\text{light efflux}}$  was greater than the rate of  $^{13}\text{A}_x$  when irradiance was less than  $800 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  but under higher irradiances, this reversed (Fig. 2c).

In *P. deltoides*, in the MC and HC treatments, the rate of  $^{13}\text{C}_{\text{light efflux}}$  was greater than  $^{13}\text{A}_x$  when the irradiance was under  $500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ ; however, when irradiance was greater than  $500 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  rates of  $^{13}\text{A}_x$  were greater (Fig. 2d, f). In the LC treatment, above the light-compensation point rates of  $^{13}\text{A}_x$  were greater than the rate of  $^{13}\text{C}_{\text{light efflux}}$  (Fig. 2b).

In the  $\text{CO}_2$ -response curves, rates of  $^{13}\text{A}_x$  were always equal to or greater than rates of  $^{13}\text{C}_{\text{light efflux}}$  across all  $[\text{}^{13}\text{CO}_2^*]$  and  $C_i$  for both species (Fig. 3). With increasing  $C_i$  the rate of  $^{13}\text{A}_x$  and  $^{13}\text{C}_{\text{light efflux}}$  approached each other in the LC and MC treatments, in *B. napus* (Fig. 3a, c). However, in the HC treatment for *B. napus* both  $^{13}\text{A}_x$  and  $^{13}\text{C}_{\text{light efflux}}$  declined with increasing  $C_i$  (Fig. 3e).

### **Contribution of xylem-transported $\text{CO}_2$ assimilation to total assimilation**

In both species, the percentage of  $^{13}\text{A}_x$  as a total contribution to photosynthesis increased with decreasing irradiance and peaked near the light-compensation point (Fig. 6a, b). In *B. napus*, the contribution of  $^{13}\text{A}_x$  to total photosynthesis increased with increasing  $[\text{}^{13}\text{CO}_2^*]$ . The highest percentage of  $^{13}\text{A}_x$  to total photosynthesis were 0.98% (LC), 4.9% (MC) and 5.9% (HC) (Fig. 6a) which occurred near the light-compensation point. The greatest contribution of xylem-transported  $\text{CO}_2$  to total photosynthesis were 0.3% (LC), 1% (MC), and 2.5% (HC), all occurring at an irradiance of  $250 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$  (Fig. 6a). In *P. deltoides* the highest contribution of  $^{13}\text{A}_x$  to total photosynthesis occurred near the light-compensation point; however, the contribution was highest in the MC treatment (8%) compared to the LC (1.6%) and HC (3.4%) treatments (Fig. 6b). The percentage of  $^{13}\text{C}_{\text{light efflux}}$  to total photosynthesis was similar to the percentage of  $^{13}\text{A}_x$  as a contribution to photosynthesis across all irradiances (Fig. S4). The highest percentage of  $^{13}\text{C}_{\text{light efflux}}$  to total photosynthesis occurred when irradiance and rates of transpiration were low (Fig. S4). The

percentage of  $^{13}\text{C}_{\text{light efflux}}$  that exited the plant compared to total assimilation was higher in *P. deltoides* compared to *B. napus* across all  $^{13}\text{CO}_2^*$  (Fig. S4).

In the  $\text{CO}_2$ -response curves, the percentage of  $^{13}\text{A}_x$  as a total contribution of photosynthesis increased with decreasing  $[\text{CO}_2]$  and peaked at the lowest  $[\text{CO}_2]$  for both species across all  $^{13}\text{CO}_2^*$ . In *B. napus* the maximum contribution of  $^{13}\text{A}_x$  to total photosynthesis was 0.7% (LC), 3.8% (MC) and 8% (HC) (Fig. 7a); while in *P. deltoides* the maximum contribution of  $^{13}\text{A}_x$  to total photosynthesis was 3.8% (LC), 7.3% (MC) and 10% (HC) (Fig. 7b). In the  $\text{CO}_2$ -response curves, the percentage of  $^{13}\text{C}_{\text{light efflux}}$  to total photosynthesis in *P. deltoides* compared to *B. napus* across all  $[\text{CO}_2]$  (Fig. S5). The highest percentage of  $^{13}\text{C}_{\text{light efflux}}$  to total photosynthesis occurred when rates of transpiration were higher and decreased with decreasing rates of transpiration and stomatal conductance (Fig. S5).

In order to determine if the  $^{13}\text{C}_{\text{light efflux}}$  could ever reach the rate of respiration in the dark ( $R_d$ ) or  $\frac{1}{2}R_d$  in the light, the rate of  $^{13}\text{C}_{\text{light efflux}}$  from all  $^{13}\text{CO}_2^*$  was plotted with the average rate of dark respiration across all  $^{13}\text{CO}_2^*$  for each species (black line) and  $\frac{1}{2}R_d$  (dotted line) (Figs. 6c, d, 7c, d) for both the light- and  $\text{CO}_2$ -response curves. In the light-response curves, the rate of  $^{13}\text{C}_{\text{light efflux}}$  is well below the rate of  $\frac{1}{2}R_d$  and  $R_d$  across all  $^{13}\text{CO}_2^*$  for both species (Fig. 6c, d). Across all  $[\text{CO}_2]$  and  $^{13}\text{CO}_2^*$  the rate of  $^{13}\text{C}_{\text{light efflux}}$  was well below the  $R_d$  and  $\frac{1}{2}R_d$  (Fig. 7c, d).

## DISCUSSION

Our method, of placing a cut leaf in a solution of  $^{13}\text{CO}_2^*$  and measuring gas exchange using a custom LI-6400 leaf chamber coupled to a tunable diode laser absorption spectroscope (TDL), allowed for real-time measurements of the efflux of xylem-transported  $\text{CO}_2$  out of the leaf in the light ( $^{13}\text{C}_{\text{light efflux}}$ ), along with calculated rates of assimilation of xylem-transported  $\text{CO}_2$  ( $^{13}\text{A}_x$ ) for both a woody species, *Populus deltoides* and a herbaceous species, *Brassica napus*. We observed  $^{13}\text{C}_{\text{light efflux}}$  and  $^{13}\text{A}_x$  across all  $^{13}\text{CO}_2^*$  (the sum of  $[\text{CO}_2]_{\text{aq}}$ ,  $[\text{HCO}_3^-]$ ,  $[\text{H}_2\text{CO}_3]$ , and  $[\text{CO}_3^{2-}]$ ) in the light- and  $\text{CO}_2$ -response curves for both species (Fig. 2, 3, 6). As expected, rates of  $^{13}\text{C}_{\text{light efflux}}$  and  $^{13}\text{A}_x$  increased with increasing  $^{13}\text{CO}_2^*$  for both species in the light- and  $\text{CO}_2$ -response curves (Fig. 2, 3). We found that xylem-transported  $\text{CO}_2$  was most important to photosynthesis when the intercellular  $[\text{CO}_2]$  ( $C_i$ ) was low, which occurred under high irradiance and low  $[\text{CO}_2]$  (Fig. 4a, b, 5a, b). Under saturating irradiance, the contribution of  $^{13}\text{A}_x$  to total photosynthesis was between 0.2% and 2.5% going from the LC to HC treatments (Figs. 6, S4). We found the highest percentage of  $^{13}\text{C}_{\text{light efflux}}$  to total photosynthesis occurred under low irradiance and low  $[\text{CO}_2]$  (Figs. S4, S5) and was between 0.2% and 6% in the light-response and 0.1% and 11% in the  $\text{CO}_2$ -response (Fig. S4, S5).

### How large is the efflux of xylem-transported CO<sub>2</sub> exiting a leaf in the light?

Xylem-transported CO<sub>2</sub> (CO<sub>2</sub><sup>\*</sup>, the sum of [CO<sub>2</sub>]<sub>aq</sub>, [HCO<sub>3</sub><sup>-</sup>], [H<sub>2</sub>CO<sub>3</sub>], and [CO<sub>3</sub><sup>2-</sup>]) must travel through the stems and/or terminal branches of a plant before it can reach the leaves. Inside the leaves CO<sub>2</sub><sup>\*</sup> travels from the small veins in the leaf to the mesophyll cells (Fig. S1) (Hanson *et al.*, 2016). Water diffusing through the leaf can travel apoplastically or symplastically (Buckley, 2015) but water must enter the symplast for CO<sub>2</sub><sup>\*</sup> to be used for photosynthesis (see Stutz & Hanson, 2019). However, what species of inorganic carbon (*i.e.* [CO<sub>2</sub>]<sub>aq</sub>, [HCO<sub>3</sub><sup>-</sup>], [H<sub>2</sub>CO<sub>3</sub>], and [CO<sub>3</sub><sup>2-</sup>]), the location and concentration of carbonic anhydrase and carboxylases, the connectivity of the atmosphere to air spaces between cells (Stutz & Hanson, 2019), and the concentration of the CO<sub>2</sub><sup>\*</sup> will greatly influence how much CO<sub>2</sub><sup>\*</sup> is used for photosynthesis. Bloemen *et al.* (2013b) hypothesized that any CO<sub>2</sub><sup>\*</sup> that reached the leaves of *P. deltoides* saplings would be inconsequential compared to the CO<sub>2</sub> entering the leaves from the atmosphere, thus, resulting in no net loss of xylem-transported CO<sub>2</sub> from leaves. However, when Bloemen *et al.* (2015) placed cut *P. deltoides* leaves either in a <sup>13</sup>CO<sub>2</sub> or KCl solution in the same Plexiglass box for a 2 hour length of time they detected that between 6% and 15% of the total assimilates in unlabeled leaves originated from xylem-transported CO<sub>2</sub> that effluxed from the labeled leaves. Additionally, Stringer & Kimmerer (1993) measured the efflux of <sup>14</sup>CO<sub>2</sub> in cut *P. deltoides* leaves and found that less than 1% of the <sup>14</sup>CO<sub>2</sub> label escaped out the leaves in the light. We also observed a small but measurable efflux of xylem-transported CO<sub>2</sub> out of cut leaves in the light across all [<sup>13</sup>CO<sub>2</sub><sup>\*</sup>] in both the light- and CO<sub>2</sub>-response curves (Fig. 2, 3). However, when compared to total assimilation the contribution of xylem-transported CO<sub>2</sub> to total assimilation or the <sup>13</sup>C<sub>light efflux</sub> to total assimilation was very low across all [<sup>13</sup>CO<sub>2</sub><sup>\*</sup>] in both light- and CO<sub>2</sub>-response curves. The highest rates of <sup>13</sup>C<sub>light efflux</sub> occurred when rates of transpiration and stomatal conductance were highest, which occurred when vapor pressure difference (VPD) and C<sub>i</sub> were lowest (Fig. 2, 3). We found that rates of transpiration, controlled by VPD, along with stomatal conductance indicated how much xylem-transported CO<sub>2</sub> exited the leaf in the light, which was also true for the dark (Stutz *et al.*, 2017).

Rates of <sup>13</sup>C<sub>efflux</sub> out of cut *Platanus occidentalis* branches were 52% lower in the light compared to the dark (McGuire *et al.*, 2009), rates of <sup>14</sup>CO<sub>2</sub> efflux out of cut *P. deltoides* leaves decreased 83% under an irradiance of 70 μmol quanta m<sup>-2</sup> s<sup>-1</sup> in the light compared to the dark (Stringer & Kimmerer, 1993). In the light, we observed 87%, 68%, and 55% declines in <sup>13</sup>C<sub>light efflux</sub> relative to dark in the LC, MC, and HC, respectively, at a transpiration rate of 2.0 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in *B. napus* (Fig. S6) and 87%, 46%, and 40% declines in <sup>13</sup>C<sub>light efflux</sub> relative to the dark in the LC, MC, and HC, respectively, at a transpiration rate of 2.3 mmol H<sub>2</sub>O m<sup>-2</sup> s<sup>-1</sup> in *P. deltoides* (Fig. S6). At

medium and high [ $^{13}\text{CO}_2^*$ ], the percentage of xylem-transported  $\text{CO}_2$  used for photosynthesis was less than at low [ $^{13}\text{CO}_2^*$ ], and consequently, the percentage of  $^{13}\text{CO}_2$  exiting the leaf was higher at the higher [ $^{13}\text{CO}_2^*$ ]. When *P. deltoides* saplings were labeled in the field 82.6% and 94.4% of xylem-transported  $\text{CO}_2$  exited the saplings in a low ( $1.4 \text{ mmol l}^{-1}$ ) and high ( $12 \text{ mmol l}^{-1}$ ) [ $^{13}\text{CO}_2^*$ ] treatments; despite providing more xylem-transported  $\text{CO}_2$  the use of xylem-transported  $\text{CO}_2$  by *P. deltoides* did not increase as much as the rate of loss (Bloemen *et al.*, 2013b). At our medium and high [ $^{13}\text{CO}_2^*$ ], we also observed an increase in  $^{13}\text{C}_{\text{light efflux}}$  that was greater than the increase in re-assimilation, indicating diminishing returns under conditions leading to a higher supply of xylem-transported  $\text{CO}_2^*$  in  $\text{C}_3$  plants, even at  $\text{C}_i$  values where photosynthesis is  $\text{CO}_2$  limited. However, Kranz-type  $\text{C}_4$  species recapture a much larger portion of xylem-transported  $\text{CO}_2^*$  for photosynthesis (Stutz & Hanson, 2019). High rates of  $^{13}\text{C}_{\text{light efflux}}$  are not limited to low rates of  $^{13}\text{A}_x$ , rather the highest rates of  $^{13}\text{C}_{\text{light efflux}}$  coincide with the highest rates of  $^{13}\text{A}_x$ . This indicates that the supply of  $\text{CO}_2^*$  for photosynthesis, which is controlled by the rate of transpiration, is critical for controlling how much xylem-transported  $\text{CO}_2$  is used for photosynthesis as well as how much is lost. Open stomata allow atmospheric  $\text{CO}_2$  into the leaf, but also let xylem-transported  $\text{CO}_2$  out.

#### ***When is xylem-transported $\text{CO}_2$ assimilation most important***

Rates of  $^{13}\text{A}_x$  were measurable in all [ $^{13}\text{CO}_2^*$ ] and increased with increasing [ $^{13}\text{CO}_2^*$ ] provided through the xylem. High rates of  $^{13}\text{A}_x$  occurred when the rate of transpiration was high and the intercellular [ $\text{CO}_2$ ] was low for both species. In the light-response curves,  $\text{CO}_2^*$  utilization followed similar patterns to  $\text{CO}_2$  originating from the atmosphere; however, in the  $\text{CO}_2$ -response curves,  $\text{CO}_2^*$  utilization was highest under low [ $\text{CO}_2$ ], indicating a balance of the supply and demand of  $\text{CO}_2^*$  for photosynthesis. Rates of  $^{12}\text{A}_{\text{obs}}$  were the same in *P. deltoides* across all [ $^{13}\text{CO}_2^*$ ]; however, in *B. napus* rates of  $^{12}\text{A}_{\text{obs}}$  were lower in the HC treatment compared to all other treatments in both the light- and  $\text{CO}_2$ -responses.

In the  $\text{CO}_2$ -response curves,  $\text{CO}_2^*$  was most important to overall photosynthesis when Rubisco was  $\text{CO}_2$  limited (Sharkey *et al.*, 2007). Rates of  $^{13}\text{A}_x$  were highest below  $200 \mu\text{mol mol}^{-1} \text{CO}_2$  when  $\text{CO}_2$  is limiting for photosynthesis (Sharkey *et al.*, 2007). We observed the peak of  $^{13}\text{A}_x$  occurring when [ $\text{CO}_2$ ] was  $100 \mu\text{mol mol}^{-1} \text{CO}_2$  across all [ $^{13}\text{CO}_2^*$ ] and both species (Fig. 3). Below this [ $\text{CO}_2$ ] there was a decline in  $^{13}\text{A}_x$  which is likely a result of deactivation of Rubisco (Sharkey *et al.*, 2007). The combination of low supply of atmospheric  $\text{CO}_2$  (low intercellular  $\text{CO}_2$ ) and high rates of transpiration and stomatal conductance supplying high quantities of [ $\text{CO}_2^*$ ] to the leaf resulted in the

observed high rates of  $^{13}\text{A}_x$  when the  $[\text{CO}_2]$  was low. As the atmospheric  $[\text{CO}_2]$  increased, the rate of transpiration supplying  $\text{CO}_2^*$  to the leaf and stomatal conductance declined. This diluted a proportion of xylem-transported  $\text{CO}_2$  in the intercellular air space and decreased the possibility of the xylem-transported  $\text{CO}_2$  being used for photosynthesis.

### ***Does xylem-transported $\text{CO}_2$ matter for photosynthesis?***

It is well established that cut branches with attached leaves, as well as cut leaves can use xylem-transported  $\text{CO}_2$  for photosynthesis (McGuire *et al.*, 2009; Stringer & Kimmerer, 1993; Bloemen *et al.*, 2015); however, when labeled xylem-transported  $\text{CO}_2$  is applied to the roots or shoots of a plant only small quantities of xylem-transported  $\text{CO}_2$  is used for leaf photosynthesis (Bloemen *et al.*, 2013a, b) or not observed in leaf tissue (Powers & Marshall, 2011). The real-time rates of xylem-transported  $\text{CO}_2$  assimilation are low compared to assimilation rates using  $\text{CO}_2$  from the atmosphere, especially when considering that most plants have  $[\text{CO}_2^*]$  in the xylem near our LC treatment. The  $[\text{CO}_2^*]$  we added to cut leaves are well within stem  $[\text{CO}_2^*]$  measured in the field for *P. deltoides*, 2.8 to 35  $\text{mmol l}^{-1}$  (Saveyn *et al.*, 2008; Aubrey & Teskey, 2009); the average branch  $[\text{CO}_2^*]$  measured in this cohort of *P. deltoides* was 2.8  $\text{mmol l}^{-1}$  (Stutz *et al.*, 2017). However, it is unknown how  $[\text{CO}_2]$  differ between the stem and branches, therefore, making it impossible to estimate how much xylem-transported  $\text{CO}_2$  maybe reaching the leaves of a large tree (Stutz *et al.*, 2017). Stem  $[\text{CO}_2^*]$  in *B. napus* was 0.7  $\text{mmol l}^{-1}$  (Stutz *et al.*, 2017) which was much lower than the  $[\text{CO}_2^*]$  in this study.

We observed a  $C_i$  difference of less than 10  $\mu\text{mol mol}^{-1}$  when accounting for xylem-transported  $\text{CO}_2$  across all treatments (Tables S1, S2). Therefore, it is unlikely that xylem-transported  $\text{CO}_2$  would significantly change our estimations of parameters for general leaf-level photosynthesis models as Hanson & Gunderson (2009) postulated. However, when considering over the life of a plant xylem-transported  $\text{CO}_2$  may play a significant role in total net photosynthesis needed for growth.

The rate of  $^{13}\text{C}_{\text{light efflux}}$  in the HC treatment was approximately a tenth of the rate of dark respiration in *B. napus* and about a fifth the rate of dark respiration in *P. deltoides* therefore, xylem-transported  $\text{CO}_2$  exiting the leaf will only affect estimates of respiration in the light for plants on the high end of measured xylem  $\text{CO}_2$  concentrations. However, if one assumes that rates of day respiration are half the rate of dark respiration, the rate of  $^{13}\text{C}_{\text{light efflux}}$  in the HC treatment would account for 40% of the efflux of  $\text{CO}_2$  out of a leaf in the light in *B. napus* and 20% in *P. deltoides*. In

the future, we would like to see how much  $^{13}\text{C}$  was acid stable to determine definitively how much xylem-transported  $\text{CO}_2$  was fixed by the leaf vs. the amount remaining as unreacted inorganic carbon. We expected most of the xylem-transported  $\text{CO}_2$  to be found in sugars based on the work by Stringer & Kimmerer (1993), that found 47.5% of the  $^{14}\text{CO}_2$  added to cut *P. deltoides* leaves was fixed by sugars in the light and that 9.4% of the xylem-transported  $\text{CO}_2$  was residual in the leaf.

## CONCLUSION

Unlike previous studies, our technique of using a LI-6400 coupled to a TDL allows for real-time measurements of the rates of xylem-transported  $\text{CO}_2$  assimilation and the efflux of xylem-transported  $\text{CO}_2$  out of a cut leaf in the light from both a herbaceous and woody  $\text{C}_3$  plant. This relies on a transpiration-based estimation of the amount of xylem-transported  $\text{CO}_2$  entering the leaf, which is straight-forward to calculate. Rates of  $^{13}\text{A}_x$  and  $^{13}\text{C}_{\text{light efflux}}$  increased with increasing  $^{13}\text{CO}_2^*$  for both the light- and  $\text{CO}_2$ -response curves. Both the woody species, *P. deltoides* and the herbaceous species, *B. napus* responded similarly to each other indicating that the effects of petiole and leaf morphology had little impact on how xylem-transported  $\text{CO}_2$  was used for photosynthesis. The highest rates of  $^{13}\text{A}_x$  and  $^{13}\text{C}_{\text{light efflux}}$  occurred when the rate of transpiration was high which occurred when irradiance was high and  $[\text{CO}_2]$  was low. The contribution of  $^{13}\text{A}_x$  to total assimilation accounted for ~2 to 10% across treatments. It is unlikely that the contribution of xylem-transported  $\text{CO}_2$  changes estimates of current models of leaf-level photosynthesis. However, assimilation of xylem-transported  $\text{CO}_2$  is likely to be important over the life of a plant and could allow plants to maintain a position carbon balance during adverse conditions.

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#### **AUTHOR CONTRIBUTIONS**

S.S.S. performed experiments and analyzed data. D.T.H. provided a conceptual framework. S.S.S. wrote the manuscript with input from D.T.H.

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#### **Supporting information (SI)**

**Table S1** Difference in internal [ $\text{CO}_2$ ] ( $C_i$ ) taking into account the addition of xylem-transported  $\text{CO}_2$  to total  $C_i$  in light-response curves.

**Table S2** Difference in internal [ $\text{CO}_2$ ] ( $C_i$ ) taking into account the addition of xylem-transported  $\text{CO}_2$  to total  $C_i$  in  $\text{CO}_2$ -response curves. Calculations are the difference between  $C_i$  not accounting for xylem-transported  $\text{CO}_2$  and  $C_i$  taking into account xylem-transported  $\text{CO}_2$ .

**Fig. S1** Detailed leaf cross section (modified from Hanson *et al.* 2016) showing  $^{13}\text{C}$  and  $^{12}\text{C}$  fluxes in our labeling experiments.

**Fig. S2** Graphical representation of equation 2, showing the calculation of the efflux of xylem-transported CO<sub>2</sub> out of a leaf in the light ( $^{13}\text{C}_{\text{light efflux}}$ ) is calculated.

**Fig. S3** Graphical representation of equation 3, showing the calculation for the rate of assimilation using xylem-transported CO<sub>2</sub> ( $^{13}\text{A}_x$ ).

**Fig. S4** Proportion of xylem-transported CO<sub>2</sub> used for photosynthesis ( $^{13}\text{A}_x$ ) and efflux in the light ( $^{13}\text{C}_{\text{light efflux}}$ ) to total assimilation in the light-responses.

**Fig. S5** Proportion of xylem-transported CO<sub>2</sub> used for photosynthesis ( $^{13}\text{A}_x$ ) and efflux in the light ( $^{13}\text{C}_{\text{light efflux}}$ ) to total assimilation in the CO<sub>2</sub>-responses.

**Fig. S6** Efflux of xylem-transported CO<sub>2</sub> from cut leaves in the dark and light.

## FIGURES

**Fig 1** Illustration of the fluxes of CO<sub>2</sub> into and out of a cut leaf in the light with inorganic carbon dissolved in the water supplied through the petiole. The water carrying dissolved inorganic carbon travels through the leaf eventually exiting the leaf as the transpiration stream (solid blue arrow). Some of the xylem-transported inorganic carbon carried with the water is used for photosynthesis by the leaf (<sup>13</sup>A<sub>x</sub>—purple dotted arrow—Equation 3) while some of the xylem-transported inorganic carbon exits the leaf as CO<sub>2</sub> (<sup>13</sup>C<sub>light efflux</sub>—red dotted arrow—Equation 2) and is measured by the TDL. Simultaneously, mostly <sup>12</sup>CO<sub>2</sub> diffuses into the leaf from the atmosphere (purple solid arrow) with a small amount of <sup>13</sup>CO<sub>2</sub> (purple solid arrow) and is used for photosynthesis. Leaf respiration also occurs in the light releasing mostly <sup>12</sup>CO<sub>2</sub> (solid red arrow).

**Fig. 2** Light-response curve for rates of xylem-transported CO<sub>2</sub> assimilation (<sup>13</sup>A<sub>x</sub>) (circles) and the efflux of <sup>13</sup>CO<sub>2</sub> exiting the leaf in the light (<sup>13</sup>C<sub>light efflux</sub>) (upside down triangles) measured under (a, b) LC (light gray), (c, d) MC (dark gray) and (e, f) HC (closed) on cut leaves of *B. napus* and *P. deltoides*. Measurements represent means and ±1 SD of five replicates for each treatment. The low-carbon (LC) was 1.19 mmol l<sup>-1</sup>, medium-carbon (MC) was 5.95 mmol l<sup>-1</sup> and high-carbon (HC) was 11.9 mmol l<sup>-1</sup>.

**Fig. 3** CO<sub>2</sub>-response curve for rates of xylem-transported CO<sub>2</sub> assimilation (<sup>13</sup>A<sub>x</sub>) (circles) and the efflux of <sup>13</sup>CO<sub>2</sub> exiting the leaf in the light (<sup>13</sup>C<sub>light efflux</sub>) (upside down triangles) measured under (a, b) LC (light gray), (c, d) MC (dark gray), and (e, f) HC (closed) on cut leaves of *B. napus* and *P. deltoides*. Measurements represent means and ±1 SD of five replicates for each treatment. The low-carbon (LC) was 1.19 mmol l<sup>-1</sup>, medium-carbon (MC) was 5.95 mmol l<sup>-1</sup> and high-carbon (HC) was 11.9 mmol l<sup>-1</sup>.

**Fig. 4** Light-response curves for rates of photosynthesis with <sup>13</sup>CO<sub>2</sub> (<sup>13</sup>A<sub>x</sub>—photosynthesis using xylem-transported CO<sub>2</sub>, or background rates of <sup>13</sup>A<sub>obs</sub> in KCl treatment) and <sup>12</sup>CO<sub>2</sub> (<sup>12</sup>A<sub>obs</sub>—photosynthesis using CO<sub>2</sub> derived from the atmosphere). <sup>13</sup>A<sub>x</sub> in (a) *B. napus* and (b) *P. deltoides*, KCl (open circles), LC (light gray circles), MC (dark gray circles), and HC (closed circles). Rates of <sup>12</sup>A<sub>obs</sub> for (c) *B. napus* and (d) *P. deltoides*, KCl (open triangles), LC (light gray squares), MC (dark gray squares) and HC (closed squares). Measurements represent means and ±1 SD of five replicates for each

treatment. The low-carbon (LC) was 1.19 mmol l<sup>-1</sup>, medium-carbon (MC) was 5.95 mmol l<sup>-1</sup>, and high-carbon (HC) was 11.9 mmol l<sup>-1</sup>.

**Fig. 5** CO<sub>2</sub>-response curves for rates of photosynthesis with <sup>13</sup>CO<sub>2</sub> (<sup>13</sup>A<sub>x</sub>—photosynthesis using xylem-transported CO<sub>2</sub> or background rates of <sup>13</sup>A<sub>obs</sub> in KCl treatment) and <sup>12</sup>CO<sub>2</sub> (<sup>12</sup>A<sub>obs</sub>—photosynthesis using CO<sub>2</sub> derived from the atmosphere). Rates of xylem-transported photosynthesis in (a) *B. napus* and (b) *P. deltoides*, KCl (open circles), LC (light gray circles), MC (dark gray), and HC (closed circles). Rates of <sup>12</sup>A<sub>obs</sub> for (c) *B. napus* and (d) *P. deltoides*, KCl (open squares), LC (light gray squares), MC (dark gray triangles) and HC (closed squares). Measurements represent means and ±1 SD of five replicates for each treatment. The low-carbon (LC) was 1.19 mmol l<sup>-1</sup>, medium-carbon (MC) was 5.95 mmol l<sup>-1</sup> and high-carbon (HC) was 11.9 mmol l<sup>-1</sup>. In the KCl treatment rates of <sup>13</sup>A<sub>obs</sub> were measured in place of rates of <sup>13</sup>A<sub>x</sub>.

**Fig. 6** Light-response curves for the percentage of xylem-transported CO<sub>2</sub> assimilation (<sup>13</sup>A<sub>x</sub>) to total rates of photosynthesis under LC (gray hexagons), MC (closed hexagons) and HC (open hexagons) for (a) *B. napus* and (b) *P. deltoides*. Light-response curves for the efflux of <sup>13</sup>CO<sub>2</sub> exiting the leaf in the light (<sup>13</sup>C<sub>light efflux</sub>) under LC (gray diamonds), MC (closed diamonds) and HC (open diamonds) for (c) *B. napus* and (d) *P. deltoides*. In plots, c and d the black line represents the average respiration in the dark (R<sub>d</sub>) across all treatments for each species, 2.07 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> and 1.56 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> for *B. napus* and *P. deltoides*, respectively. While the dotted lines represent ½\*R<sub>d</sub> averaged across all treatments for each species, 1.04 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> and 0.78 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup>, for *B. napus* and *P. deltoides*, respectively. Measurements represent means and ±1 SD of five replicates for each treatment. The low-carbon (LC) was 1.19 mmol l<sup>-1</sup>, medium-carbon (MC) was 5.95 mmol l<sup>-1</sup> and high-carbon (HC) was 11.9 mmol l<sup>-1</sup>.

**Fig. 7** CO<sub>2</sub>-response curves for the percentage of xylem-transported CO<sub>2</sub> assimilation (<sup>13</sup>A<sub>x</sub>) to total rates of photosynthesis under LC (light gray hexagons), MC (dark gray hexagons) and HC (closed hexagons) for (a) *B. napus* and (b) *P. deltoides*. Light-response curves for the efflux of <sup>13</sup>CO<sub>2</sub> exiting the leaf in the light (<sup>13</sup>C<sub>light efflux</sub>) under LC (light gray diamonds), MC (dark gray diamonds), HC (closed diamonds) for (c) *B. napus* and (d) *P. deltoides*. In plots, c and d the black line represents the average respiration in the dark (R<sub>d</sub>) across all treatments for each species, 2.08 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> and

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1.86  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  for *B. napus* and *P. deltoides*, respectively. While the dotted lines represent  $\frac{1}{2}R_d$  averaged across all treatments for each species, 1.04  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  and 0.93  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ , for *B. napus* and *P. deltoides*, respectively. Measurements represent means and  $\pm 1$  SD of five replicates for each treatment. The low-carbon (LC) was 1.19  $\text{mmol l}^{-1}$ , medium-carbon (MC) was 5.95  $\text{mmol l}^{-1}$  and high-carbon (HC) was 11.9  $\text{mmol l}^{-1}$ .

## TABLES

Table 1 Definitions and units for symbols in the text.

All units are  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ .

Symbol	Definition	Equations/notes
$^{12}\text{A}_{\text{obs}}$	Net $^{12}\text{CO}_2$ assimilations	Measured with the TDL
$^{13}\text{A}_{\text{obs}}$	Net $^{13}\text{CO}_2$ assimilations	Measured with the TDL
$^{13}\text{A}_{\text{pred}}$	Predicted atmospheric $^{13}\text{CO}_2$ assimilation	Equation 1
$^{13}\text{C}_{\text{dark efflux}}$	Efflux of $^{13}\text{CO}_2$ in the dark.	Measured with the TDL See Stutz <i>et al.</i> 2017
$^{13}\text{C}_{\text{pred efflux}}$	Predicted rate of xylem $^{13}\text{CO}_2$ efflux assuming no xylem transported $\text{CO}_2$ is used for photosynthesis	See Stutz <i>et al.</i> 2017
$^{13}\text{C}_{\text{light efflux}}$	Calculated $^{13}\text{C}_{\text{efflux}}$ in the light	Equation 2
$^{13}\text{A}_x$	Assimilation of xylem transported $\text{CO}_2$	Equation 3

Fig. 1













